PUBLICATION 475 ISSUED AUGUST 1935
TECHNICAL BULLETIN 1 FIRST PRINTING
DOMINION OF CANADA - DEPARTMENT OF AGRICULTURE

THE EFFECT OF FEEDING DEAMINIZED VERSUS UNTREATED COD LIVER OILS ON GROWTH, EGG PRODUCTION AND MORTALITY OF POULTRY

H. S. GUTTERIDGE

DIVISION OF POULTRY HUSBANDRY
EXPERIMENTAL FARMS BRANCH

Published by authority of the Hon. ROBERT WEIR, Minister of Agriculture
Ottawa, Canada

DOMINION EXPERIMENTAL FARMS

E. S. Archibald, B.A., B.S.A., LL.D., D.Sc., *Director*

F. C. Elford, *Dominion Poultry Husbandman*

George Robertson
Chief Assistant

A. G. Taylor, B.S.A.
H. S. Gutteridge, B.S.A., M.Sc.
Poultry Husbandmen

TABLE OF CONTENTS

4761—1½

The Effect of Feeding Deaminized versus Untreated Cod Liver Oils on Growth, Egg Production and Mortality of Poultry.[1]

H. S. Gutteridge[2]

I. INTRODUCTION

During the period from 1922 to 1928 the use of vitamin supplements in poultry rations became common practice throughout the world. With the accumulation of knowledge as to the functions of the vitamins and the necessity for vitamin prophylaxis, it became readily apparent that they were particularly necessary in the feeding of poultry in view of the unnatural environmental conditions which a specialized industry imposed upon them. In addition, research with chickens as experimental subjects indicated a much greater requirement for most of the vitamins than was the case with mammals, upon which species practically all previous vitamin research had been carried out. Since it early became apparent that vitamins A and D were required in relatively large amounts and since the average poultry ration was very likely to be low in these two vitamins, particularly during the winter months or under conditions of confinement, a supplement containing both of these vitamins was fed. Cod liver oil thus became a standard ingredient of poultry rations as being the cheapest and most efficient source of these vitamins.

Coincident with the conditions of environment and cod liver oil feeding noted above, certain abnormalties appeared in poultry flocks which had not been previously noticed particularly, and which might have been attributed to either unnatural environmental conditions, to the demands which increasingly higher egg production made upon the birds, or to other numerous but apparently less likely causes. The abnormalties referred to include cannibalism, feather pulling and a greatly increased mortality both during the growing and egg production periods, but particularly during the latter.

While a study of these factors (unnatural environment and high production) obviously was a very logical line of attack upon this problem, it occurred to the writer that the coincidence of cod liver oil feeding and the increase of these abnormalties was not entirely a matter of chance but might afford an avenue of approach to the solution of the problem which was also worthy of investigation. The decision to put this possibility to the test was further strengthened by the fact that even up to that time (1933) the quality of cod liver oils available for poultry use left much to be desired, varying from those steam-rendered from fresh livers to others produced by the sun-rotting process in which oil and decomposed liver were intimately mixed.

A. Impurities of Cod Liver Oils

Cod liver oils are fed to poultry solely as a source of vitamins. Hence, all products other than the vitamins themselves and the pure oil, which of necessity is their carrier, may be considered as impurities. The presence of an additional 1 or 2 per cent of fat in the ration, as pure fish oil, may be considered to have a negligible effect upon the final result since poultry have been shown on numerous occasions to have a considerable fat tolerance, although it was at

[1] Contribution from the Division of Poultry Husbandry, Central Experimental Farm.
[2] Poultry Husbandman.

one time considered that the digestibility of this nutrient was not high. More recent work, however, has shown fats to be relatively highly digestible to poultry (Fraps, 1928).

The principal impurities of most cod liver oils, and the ones of most importance from the standpoint of this study, are free fatty acids and any dissociation products of liver material, which, either through lack of proper care in handling remain with the suspended liver material in the oil, or are water or fat soluble and thus present in solution. These will be considered briefly in the order named.

(1) Free Fatty Acids.—All cod liver oils contain free fatty acids to a greater or lesser degree, those being used for medicinal purposes containing usually under 1 per cent, expressed as oleic acid. Poorer grade oils run much higher in fatty acid levels, actually up to 30 per cent, as will later be pointed out. It is an established fact that oil of low free fatty acid content, upon standing under sub-ideal conditions of storage, increases in free fatty acid content. It is also established that free fatty acid content is higher in oil made from stale livers than is the case when fresh livers are used.

In a study such as is here undertaken the factor of greatest importance is not the nature of the impurity but its biological effect. In the case of free fatty acids a very definite opinion existed for some years, based mainly upon observation, that cod liver oils of high free fatty acid content were deleterious when fed to chicks, as judged by lowered growth, lack of uniformity and high mortality. That this conclusion rested on a sound basis was shown by Holmes et al (1930) when they demonstrated that oils chosen from the market and containing 5·92 per cent and 11·65 per cent of free fatty acids, respectively, gave a markedly decreased growth from that obtained from comparable chicks receiving an oil of 0·98 per cent level and from the control which received pure oil of vegetable origin of 0·05 per cent free fatty acid content. The chicks which were fed the high fatty acid oils were also less uniform, and at the end of fourteen weeks were deemed not suitable to be carried to maturity. Likewise, mortality was excessively high in these groups, being 40 and 28 per cent respectively for the pens receiving 5·92 per cent and 11·65 per cent free fatty acid oils. It might be pointed out that one hundred individuals of mixed sexes were used in each lot, which number should be sufficient to largely eliminate the factor of chance from the results, although statistical measures were not applied to the data by the authors. The two oils used were very dark red in colour and of decidedly unpleasant odour and were "without doubt prepared from more or less decomposed livers." It is interesting to note, nevertheless, that they were purchased on the market for poultry feeding purposes and probably were oils produced by the sun-rotting process referred to previously. The authors make the interesting observation from an analysis of many samples, that "there is no apparent relation between the colour of an oil and its acid content."

In considering the results obtained by these investigators, it is apparent that these oils of high free fatty acid content were decidedly deleterious to chicks. That this effect was due to the free fatty acid content of these oils, does not follow, however. It is suggested, in view of information to be reviewed or reported in this bulletin, that these results were due, not to the relatively high level of free fatty acids but to certain other impurities which form the basis for the experiments here reported. It might be noted in passing that the only inconsistency in the results reported in the paper by Holmes et al (1930) under discussion, namely, a much higher level of mortality for the oil having the lower free fatty acid content (5·9 per cent) is quite logically explainable upon this basis.

In support of the contention that free fatty acidity is not responsible for such deleterious effects, *per se*, there exists the research of several investigators, notable among which is that of Hunter et al (1931) in which the free fatty acid

content of the cod liver oils used could not be correlated with the development of the young chicks. Previous work with chicks (Gutteridge 1932) also suggested that there was no retarding effect when fatty acid (oleic) was added to a chick ration in varying percentages.

There has also been accumulating, during the past few years, a rather voluminous literature indicating that fats are essential to growth in rats and that certain fatty acids, notably linoleic and linolenic, the former of which is definitely known to occur in cod liver oil, are capable of substituting for fats in a fat deficient diet, with the consequent prevention of the fat deficiency symptoms. A review of this phase of the literature, however, would be out of place in this discussion.

(2) Products of Protein Decomposition.—Since cod liver oil is in itself mainly a mixture of neutral glycerides, any impurities of protein origin must come originally from the livers and exist either as suspended liver material, in the so-called "foots" or liver material often found at the bottom of a container of cod liver oil, in solution in the oil itself, or in water which is always present to a greater or lesser extent. In addition, it contains some phospholipins such as lecithin and consequently choline as well as various sterols (Norris and Church 1930).

Gautier and Mourgues (1888) found cod liver oil to contain several nitrogenous bases together making up 0·2 per cent of the oil, namely, butylamine, isoamylamine, hexylamine, dibydrolutidine, asellin, morrhuin and morrhuic acid. Of these, isoamylamine made up one-third of the bases present and was very poisonous in nature producing rigour, convulsions and death, in a greenfinch in three minutes. Three milligrams of asellin HCl, killed a greenfinch in 14 minutes. Hawk (1907), confirmed the work of the above authors finding 1·06 to 1·17 grams of nitrogenous bases per kilo of oil. They were able to identify butylamine, isoamylamine, hexylamine, dibydrolutidine and morrhuin, but not asellin.

Norris and Church (1930) confirmed the poisonous effect of isoamylamine and choline HCl when injected into the white rat. This fact was further confirmed by the writer for isoamylamine, an injection of 7 milligrams into sparrows producing convulsions in two minutes and death in six. Norris and Church (1930) further demonstrated, by feeding to rats, orally, such a quantity of isoamylamine in oil, daily, that Gautier and Mourgues' ratio of this base to oil was approximated, that loss in weight occurred and that, when four times this amount was fed, convulsions and paralysis resulted during the last two weeks of an eight weeks' test period. The addition of 18 per cent of yeast to the ration overcame this effect although 10 per cent was ineffective.

In connection with the presence of choline in cod liver oil, a base also shown to be definitely toxic by Norris and Church (1930), it is interesting to note that out of three derivatives of choline, viz. neurine, muscarine and betaine, the first two are intensely poisonous (Winter Blyth 1920). Neurine is a product of the decomposition of choline while muscarine is derived from it by oxidation; hence, the possibility of their presence in cod liver oil would seem to be entirely likely.

Further references relating to the toxic properties of cod liver oils, in which definite fractions or impurities of the oils are specifically dealt with as being the agencies responsible for the biological effect produced, are lacking in the literature. There are, however, many papers dealing with biologically measured toxic properties of unknown nature in cod liver oils.

Slagswold (1925), for instance, found that certain cod liver oils were definitely poisonous to calves. His port-mortem findings are of interest and are noted briefly as follows: the presence of a serous exudate in the pericardium; the heart muscles pale and waxy in appearance; a reddish serous exudate in

the lungs and thoracic cavity; the kidneys congested and enlarged. The property of the oils used, which was responsible for the toxic effect, was not determined, however.

One of the earliest and most extensive series of experiments dealing with the poisonous properties of cod liver oils was that of Agduhr, reported in a series of papers during the years 1926-29. He found the cod liver oils used to be very definitely toxic to mice. Not only that, but he was able to separate from the cod liver oil "substances which proved to be very poisonous to the organism. As an example, it may be mentioned that, from 200 c.c. of cod liver oil, extracted substances of this kind have, after neutralization of the extracting fluid, been amply sufficient to kill five white mice in good health within a day." His autopsy findings have much in common with those of Slagswold (1925), particularly in so far as the heart condition is concerned, with pigment atrophy and acute waxy (hyalenoid) degeneration of the cardiac muscles. He also notes that, through the use of certain solvents, he was able to produce oil, almost, if not entirely, free from these substances. A very interesting observation was made to the effect that the same oils were only very slightly toxic to white rats and that a great deal of variation in response to dosage of toxic oils exists with individuals within the species as well as between species.

Harris and Moore (1928) found that rats receiving 15 per cent of cod liver oil grew at a much slower rate than did controls receiving 15 per cent of arachis oil and that the coats of these individuals were very much rougher than the controls, a condition also found by Slagswold (1925) in his calves.

No specific toxic agent could be demonstrated in cod liver oil by Bell, Gregory and Drummond (1933) although 15 per cent of cod liver oil in the diet failed to give normal growth with rats. They state that the condition could not be due to excess of vitamins and that fresh, oxidized and sun-rotted oils gave the same results. The possibility suggests itself, in this instance, that the steam-rendered oil may have been obtained from livers not strictly fresh and that the rats, being less sensitive diagnostic subjects, as shown by Agdhur (1927), small differences in toxicity could not be measured.

Yamamoto (1934) verified the findings of previous workers in that 10 to 15 per cent of cod liver oil was toxic to rats and that the condition was alleviated by large amounts of yeast. Substitution of butter or olive oil for the cod liver oil gave improved results. He concludes that the toxic effect is not related to hypervitaminosis but is associated with fatty acid content.

It would appear from the above review that several workers are agreed upon the fact that certain cod liver oils have a definitely toxic effect. No explanation for the counteracting effect of yeast has been advanced although it is clear that a deficiency of the vitamin B complex does not enter into the syndrome since very greatly excessive quantities of yeast are required to prevent the appearance of the condition. The possibility of the existence of hypervitaminosis A or D is disclaimed by several workers and could not be the case with others in whose experiments only normal levels of cod liver oil were used. It might be pointed out that in routine testing work the writer has found that 2, 3 and 4 per cent levels of feeding with certain oils gave lower growth than the normal 1 per cent levels (Gutteridge 1931), a circumstance which contributed to the desire to investigate the problem herein reported.

(3) Relation of Free Fatty Acids to Products of Protein Decomposition.—It is to be noted that two of the experiments above reviewed relate the toxic properties of certain cod liver oils to their fatty acid content. In neither case was an attempt made to determine the effect of free fatty acids, in themselves, upon the well-being of the subjects. Neither was any attempt made to purify the oils of any other substances which might possibly be of a

toxic nature. In the case of the first reported work, namely, that of Gautier and Mourgues (1888), there is no doubt that the oil used was of a very crude nature since methods of refining as we now know them are of quite recent development. The work of both Agduhr (1926, 1927) and of Slagswold (1925) may be considered in the same light but probably to a lesser degree, while the oils used by Holmes et al (1930) were admittedly of a crude nature. Hence, it is probable that in all these instances contamination with rotted liver material or preparation from stale livers or both were factors in the production of the toxic condition encountered. Consequently both high free fatty acidity and a concentration of protein decomposition products were possible in these instances.

In connection with the data here to be reported analyses were made of a large group of oils of different types and from different sources both for content of free fatty acids and of nitrogen. The data of table 1 (text) are given as indicative of the relationship existing between these two fractions of cod liver oils.

TABLE 1.—RELATION BETWEEN FREE FATTY ACID AND NITROGEN CONTENT, IN FISH OILS

Sample No.	Description	Free fatty acids as oleic	Grams nitrogen per 1,000 c.c. of oil	Ratio of free fatty acid to nitrogen
		%		
1	Medicinal cod liver oil (Newfoundland)	0·25	0·0051	49·0
2	Medicinal cod liver oil (Norwegian)	0·30	0·0030	100·0
3	Pilchard oil (feeding purposes)	0·60	0·0081	74·1
4	Concentrate cod liver oil (feeding purposes)	3·17	0·0240	132·1
5	Steam rendered cod liver oil (feeding purposes)	11·95	0·0119	1,004·2
6	Steam rendered cod liver oil (feeding purposes)	21·7	0·0490	442·8
7	Sun rendered, cod liver oil (feeding purposes)	29·6	0·3100	95·5

The data of table 1 indicate very definitely that a relationship exists between free fatty acidity and the nitrogen content of fish oils. Although the free fatty acidity increased from 0·25 to 29·6, or 118·4 times, the ratio of free fatty acid to nitrogen remained reasonably constant. Actually, nitrogen content also increased from the lowest to the highest level by 103·3 times. That this constancy of ratio is not necessarily always the case is indicated by the fact that oils Nos. 5 and 6 showed a proportionately greater ratio of free fatty acids to nitrogen than did any of the other oils. As noted in the table these are the only high free fatty acid oils which were steam rendered. It would seem that the process of steam-rendering produces an oil of lower nitrogen content than does sun-rendering, as would be expected, since in sun-rendering the livers are allowed to rot in puncheons for some time, the oil rising to the surface. The concentration of liver material in the oil and the quantity of by-products of protein decomposition would logically be expected to be higher under this treatment. Pilchard oil, a body oil, is remarkably low in both fractions. It is interesting to note that a very high free fatty acid oil, such as No. 6 with 21·7 per cent free fatty acid, can be obtained even though steam-rendered. In explanation of this fact the manufacturer stated that while this oil had been carefully steam-rendered, to his knowledge it had been made from stale livers. It would appear, therefore, that both free fatty acids and nitrogen may be high unless the livers used are fresh at the time of rendering.

Since all of the above were oils typical of those actually sold on the market and were from widely varying sources it may be said that a strong tendency exists for high free fatty acid oils to be also high in nitrogen, although this is not necessarily the case. The relationship between the nitrogen content and the

presence of nitrogen compounds (amines) in which it occurs is a very direct one, as will be appreciated from the discussion of this matter earlier in this bulletin.

Since it seemed logical to use an oil high in nitrogen content in attempting to measure the biological effect of the nitrogenous fraction, cod liver oil No. 7 was used throughout the experiments here reported. It should be said that this oil was medium dark in colour and not particularly strong or fishy in odour. In these respects it might be said to be, in the author's experience, a sun-rendered oil of better than average quality.

Judging from the information just reviewed, it seemed to be apparent that sufficient grounds existed to presume that some cod liver oils contained appreciable quantities of nitrogenous bases of an intensely poisonous nature. The definitely toxic effect of the products themselves and of the oils in which they were found was demonstrated with rats and mice at least, and with small chicks as well, if it be presumed, as appears to be the case, that free fatty acids of these oils are themselves not harmful. It was, therefore, decided to test the possible harmful effects of these nitrogenous bases upon growth, egg production and mortality when fed to chickens, in the oils in which they occurred, at normally recommended levels of feeding of cod liver oils.

II. EXPERIMENTAL

The methods of Gautier and Mourgues (1888) for the isolation and quantitative determination of the nitrogenous bases which they identified were critically examined with a view to determining the quantitative occurrence of the nitrogenous bases in cod liver oils. It proved impossible, however, to duplicate their methods in a satisfactory manner, due chiefly to the difficulty in following the procedures as outlined by them and to the further fact that advances in quantitative determination since that time have indicated that too much reliability could not be placed upon the results of such determinations when carried out.

A. Chemical Processing of Oils

Since these bases were all protein decomposition products, and therefore nitrogenous in nature, it was thought that a procedure might be established which would satisfactorily remove these substances from cod liver oils without interfering with their content of vitamins A and D, the former of which is relatively unstable, particularly to oxidation. The following process was tentatively adopted, subject to its justification by a vitamin test: equal parts by volume of cod liver oil and ethyl ether, with two parts by volume of 10 per cent sulphuric acid, mixed and shaken thoroughly at half-hour intervals covering a period of four hours. The mixture is left over night and then washed with distilled water until free from sulphates as indicated by the barium chloride test of Hawk and Bergeim (1927). The ether is then distilled off under reduced pressure (27 inches of mercury under negative pressure) at a temperature of 38° C., until the oil is entirely free from the odour of ether, a process requiring three to four hours.

It was considered that this process had removed entirely the nitrogen bases present, and it was thought to be most correctly termed a process of deaminization. Hence, oils so treated will be referred to in this bulletin as deaminized oils. It was ascertained that any injury to the vitamins which may have been brought about by this process was negligible in its biological effect through the carrying out of biological tests for both vitamins A and D, according to a technique reported elsewhere (Gutteridge 1931, 1932), the line test (Miller et al

1929) being used as the criterion for sufficiency of vitamin D and growth and absence of typical deficiency symptoms and the post-mortem presence of excessive urates, for vitamin A.

B. The Effect of Deaminization of Oils Upon Production of Growth

For this experiment, an entire hatch of five hundred and sixty-three cross-bred White Leghorn male x Barred Rock female chicks was used. These were separated according to sex, at hatching time, by rate of feather growth (Warren 1930). An excellent hatch was obtained and the chicks were particularly uniform and vigorous. Since it was essential that they be divided as evenly as possible between the two experimental lots, they were taken from the incubator in baskets, each chick banded and weighed and placed in one or other of the two experimental lots, alternately as they came to hand. In this way it was felt that any bias in favour of one pen or the other would have little chance of existing, since reasonably good randomization should be effected when such large numbers were being dealt with. As a result of this division there existed two experimental lots of chicks, one designated as lot A, containing 284 individuals equally divided as to sex, and another designated as lot B containing 279 chicks also equally divided as to sex. These lots formed the basis for the experiments for both growth and egg production, pen A receiving the untreated oil in every instance and pen B the deaminized oil.

(1) The Brooding Period (1 to 8 Weeks).—The two lots of chicks, A and B, were distributed over sixteen compartments of a battery brooder, lots from the two pens alternating throughout and each compartment containing approximately equal numbers of cockerels and pullets. The same well-balanced ration was fed to each lot and was composed of the following ingredients by weight:—

Wheat bran	11
Alfalfa leaf meal	11
Wheat middlings	22
Yellow corn meal	22
Ground oat groats	22
Meat meal	6
Fish meal	2
Buttermilk powder	2
Bone meal	2
Total	100

To this mash was added 0·5 per cent of common salt.

The rations given to lots A and B were identical, with the exception that 1 per cent by weight of unprocessed cod liver oil was added to the mash given to lot A, whereas an equal quantity of the same oil, previously deaminized, was added to the mash given to lot B. These additions were made to the mash when fresh lots were mixed from time to time, so as to limit deterioration of the oil in the mixed feed to as great an extent as possible. These mashes were before the chicks at all times.

All chicks were weighed individually each week, and the feed consumption was recorded at that time. It should be noted that the data for feed consumption during the period of growth were not used in the determination of the efficiency of these feeds, owing to the fact that unavoidable wastage occurred. The matter of obtaining accurate feed consumption in battery tests presents a great many difficulties, owing to the apparent facility with which chicks flip mash around and carry it to the drinking vessels. The rough figures obtained, however, were sufficient to indicate that both mashes were being avidly consumed. This would be expected, since the only difference was the oil itself, which, at a 1 per cent level of the total mash mixture, is probably a negligible factor from the standpoint of palatability.

Table 1 (Appendix A) shows the complete data for this period for the total population of mixed sexes.

A study of this table indicates that, in spite of the fact that at the start the chicks in pen A proved to be heavier than those in pen B, the chicks of the latter pen were the heavier throughout with the exception of the first two weeks. The data indicate, then, that the removal of nitrogenous impurities from the oil permitted growth superior to that of the control chicks receiving the same oil from which this fraction had not been removed.

Mortality during this period was 20 and 15 individuals, or 7·0 per cent and 5·4 per cent for pens A and B, respectively. Since it was not possible through post-mortem examination to determine the exact cause of death or to relate autopsy findings in any way to treatment of the chicks, the possible importance, or otherwise, of this difference cannot be judged.

(2) The Brooding Period (1 to 8 Weeks), Males and Females Separated).—Since it was not possible to carry the full flock to maturity, the population was reduced by the following procedure. The χ^2 test showed that the distribution of the birds in all pens closely approached normal. Accordingly, a proportionate number of individuals was chosen from each body weight class so as to make a reduced population of both males and females, parallel in distribution to that of the original large population. It is believed that reduced populations closely representative of the original populations from which they were drawn were secured by this treatment. The males of these populations were not carried further but the pullets were retained and carried to maturity.

Table 2 (Appendix A) shows the details, for males and females separately for the reduced populations to eight weeks of age.

It will be noted from a study of the data of this table that with the exception of hatching weights the males of pen B were heavier than those of pen A in every case.

A comparison of the two groups of females, however, tells a somewhat different story. With the exception of the second week, when a significant difference existed in favour of pen A, no differences of any account occurred between the two pens, although during the last four weeks the pen B females were heavier.

It may be considered, therefore, that the presence of a nitrogenous fraction in the cod liver oil definitely retarded the growth of the males, but had little if any effect upon the females. Actually, at eight weeks of age, the males of pen A were only 3·5 per cent heavier than their females, whereas those of pen B were 6·5 per cent heavier, which relationship is approximately that expected for chicks of this cross at eight weeks. A more complete discussion of the matter of variation of sex difference may be found in a paper by Bird and Gutteridge (1934).

(3) The Rearing Period (9 to 24 Weeks), Females Only.—In Table 3 (Appendix A) are set forth the body weight data of the retained females for what is considered to be the rearing period in this instance, namely, nine to twenty-four weeks, inclusive. At eight weeks of age the pullets were transferred to two pens in a long laying house and kept confined. No changes were made in the ration.

It will be noted from Table 3, (Appendix A) that with the females the same condition as that existing prior to nine weeks holds, namely, that in practically every instance the chicks in pen B were heavier than those in pen A. Through the application of the binomial method (Miles 1935) a highly significant difference in body weight is found in favour of this lot.

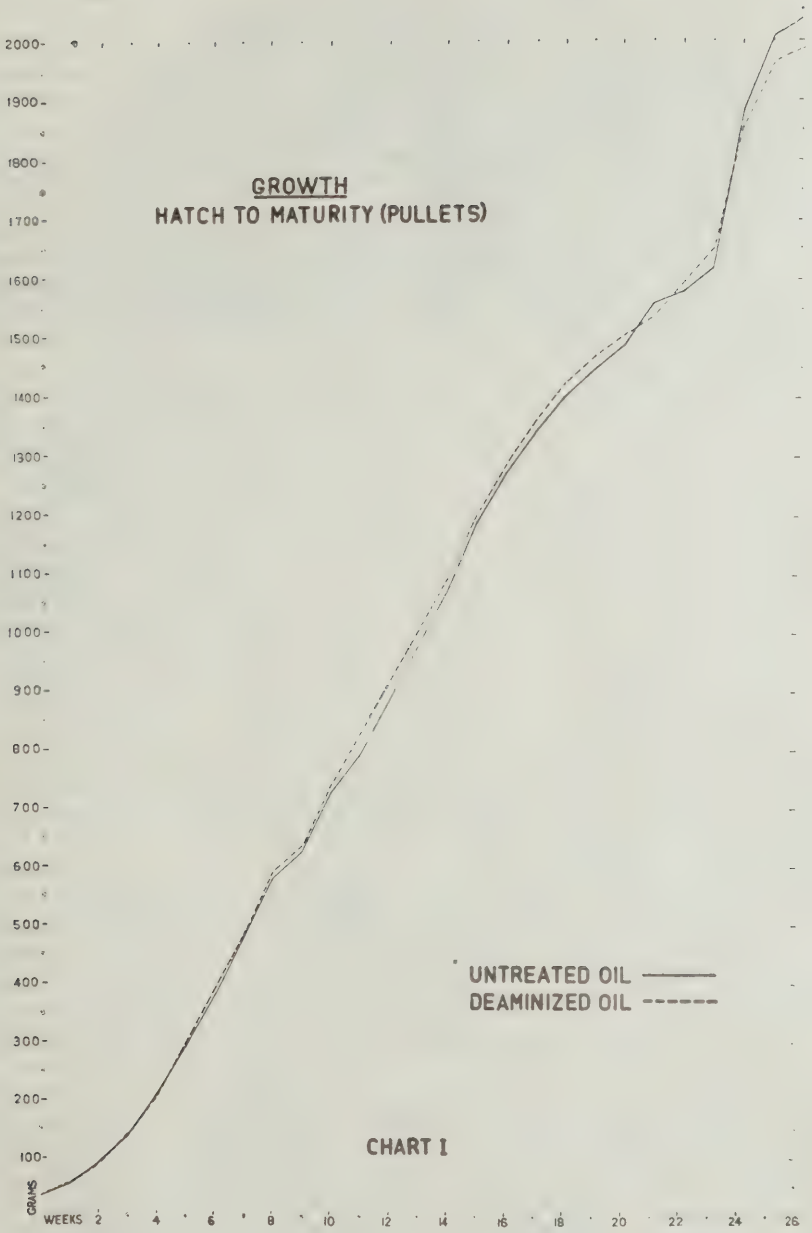
GROWTH
HATCH TO MATURITY (PULLETS)
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
GRAMS
WEEKS 2 4 6 8 10 12 14 16 18 20 22 24 26
UNTREATED OIL
DEAMINIZED OIL

CHART I

Chart No. 1 indicates very clearly the relationship existing between the two pens, in so far as body weight was concerned, during the entire period of growth. It is particularly interesting to note the reaction of these birds to environmental conditions, and, in this instance, the difference between the two pens in this regard. At nine weeks of age, coincident with removal from the battery to the laying house, a decided setback in growth was noticeable which was equally pronounced for both pens, caused by lowered feed consumption through unfamiliarity with their surroundings. At ten weeks of age, the chicks in pen A contracted bronchitis the effect of which was very noticeable at the eleventh week's weighing. Those in pen B also contracted bronchitis almost at the same time, and although both pens experienced an equally severe outbreak as judged by available symptoms (namely, the percentage of the flock affected, the degree of listlessness, the number of drooping postures and the difficulty of breathing, with rattling in the throat) the chicks in the latter pen completely recovered without having experienced any appreciable setback in body weight and recovered more rapidly. The actual figures (Table 3, Appendix A) show a slight setback for pen B, as well, but not to a sufficient extent to be apparent on the chart.

It is an established fact that just prior to sexual maturity and the commencement of laying, pullets gain in body weight very rapidly, due, without doubt, to the greater physiological activity coincident with the developing function of the reproductive organs. It was apparent from observation, and is borne out by the growth curve, that the birds in pen A started to come rapidly to maturity between the twentieth and twenty-first weeks. An examination of the individual records, shows birds of both pens coming to sexual maturity at this time, but many more of A than of B. The high body weight suddenly attained by pen A at the end of the twenty-first week occurred on the fifth of December. On the seventh of December the temperature began gradually to drop from a minimum of 18° F. above zero on that date to –14° F. (below zero), –23° F., –23° F., –13° F., –15° F., –15° F. and –12° F. on the ninth, tenth, eleventh, twelfth, thirteenth, fourteenth and fifteenth of December, respectively. That the low temperatures affected those birds severely was evidenced by their conduct and by the break in the growth curve from the twenty-first to the twenty-third weeks. Pen B, although equally exposed and with an appreciable number of females coming to maturity, actually increased its rate of growth during the same weekly periods.

The twenty-third week witnessed a suddenly improved growth rate for both pens, fostered, no doubt, by the transference of the birds to individual pens in a heated house at that time. The growth curves also indicate that the birds in pen A attained a greater weight than those in pen B at maturity, a weight difference which persisted, though to a lessened degree, throughout the egg production period. The matter of sensitivity to environmental conditions and of the greater mature weight will be more fully discussed later in this bulletin.

It has been the author's experience over a period of years that with growing chicks good gains are almost invariably uniform gains. The same principle has been enunciated by numerous others as an example of which the report of Mitchell and Grindley (1913), based on experience and experiment with larger farm animals, may be noted. The weekly variability of both pens from time of hatch was calculated and is set forth in Table 4 (Appendix A). A perusal of the data of this table indicates a definite superiority for pen B, uniformity being greater for every week for this pen. The application of the binomial method, while hardly necessary in such an extreme case, where all weeks are in favour of one pen, indicates a very significantly lower variability for pen B.

The difference in mortality was again insignificant during this period (9 to 24 weeks). Actually, out of 91 birds in pen A, 19, or 20·9 per cent, died, whereas out of 89 in pen B, 17 died, or 19·1 per cent.

C. Period of Egg Production

At twenty-three weeks of age the pullets of both pens were moved to individual hen batteries, having a separate compartment for each bird. All birds were then leg-banded and placed in the cages in such a way that every second bird belonged to the same lot. It was thought that allotment by such alternation of birds would tend to reduce the possibility of the effect of environmental factors being unequal for the pullets on the two treatments. Weekly body weights and feed weights were taken, as was previously done, but feed consumption in this instance was kept separately for each individual. All eggs laid were weighed individually and credited to the pullet producing them. Any eggs which were broken could not be eaten by the birds because they passed through the coarse wire floor immediately upon breakage. These were credited to the bird laying them, at the average egg weight for that bird, for the week during which they were laid.

For the period of egg production the feed was changed to a mash of the following formula:—

Ground yellow corn	46.27
Ground oat groats	11.70
Wheat bran	10.81
Wheat middlings	10.81
Alfalfa meal	7.71
Bone meal	1.80
Buttermilk powder	1.80
Fish meal	1.80
Meat meal	1.80
Salt (Nacl)	0.89
Molasses	3.61
Cod liver oil	1.00
Total	100.00

Previous tests, for the purpose, indicated that less wastage occurred if the mash was made into pellet form, which was done in this case. The molasses of the above mash was included for this purpose, and the cod liver oil was added to the mash for each pen after the heating process was completed.

It should be noted here that production was excellent in both pens throughout the test. Not only that, but the fact that the environment of the birds was controlled to such an extent that variability in production from bird to bird was greatly reduced was apparent as indicated by the fact that the average variability in number of eggs laid per bird was 14·8 per cent (both lots combined) as against a variability of 26·8 per cent for a comparable lot of fifty-two pullets of the same cross which had been kept under the conditions of an ordinary laying house. The value of individual caging in research work is well evidenced by this difference since by reference to a suitable table (Bird and Gutteridge 1934) it may be seen that, given variability of the above levels, 68 birds only would be required in each lot to demonstrate a difference of 5 per cent between treatments to be significant, whereas 222 birds per pen would be required for the same purpose under ordinary laying house conditions. The figure of 25·8 per cent is not unusual and may be considered to be but little above the average for laying house conditions in most poultry areas. It is important to note that the control of environment to this extent gives much greater opportunity for the treatment under test to show its physiological effect than would otherwise be the case.

The criteria upon which an analysis of the results during the period of egg production will be based are the average body weight which had to be maintained, the average gain or loss of body weight, the average consumption of feed and the average total weight of eggs produced. The average number of eggs laid and their average weight will also be referred to in the analysis, but the final expression of production will be considered to be the total weight of eggs produced. The reason for this should be fairly obvious in that a definite weight of feed is consumed and is used for purposes of maintenance of body weight, towards the laying on of additional body weight, and for the production of a definite weight of egg material. The actual number of eggs and the average egg weight have no significance in the measuring of the efficiency of the feed except as their product represents the total production of egg material.

(1) Total Weight Production of Eggs per Bird—weekly averages.—Table 5 (Appendix A) shows the average weekly weight production of eggs for each pen for the forty-four weeks of the production period. These data are calculated only on the records of birds which lived throughout the test.

Since the forty-four variates for each pen in this table are not independent variables but are repeated observations on the same birds, subject to a definite trend in keeping with the normal curve of egg production, which in turn is dictated by environmental and physiological considerations, it was felt that the binomial method would be the most adequate method of statistical treatment of these data. When analysed by this method, no significant difference was found to exist, the actual probability of occurrence of such a difference by chance alone being 32 in 100. Stated otherwise, no significance could be attached to the fact that pen A exceeded pen B in production on four more occasions than pen B exceeded pen A.

An interesting point with regard to experimental technique was brought to light at this time. It has been reported by Buckner et al (1934) that, whether growth data are calculated on the basis of the number of birds alive at each weighing or simply by using the records of only those which lived throughout the experiment, makes only an insignificant difference to the results. That this principle does not necessarily hold in egg production is indicated by the results of this test. When the birds which died were not eliminated from the records but included up to the time of death, lot A was superior to B in production, thirty-one weeks out of forty-four. The variation from expectancy (22:22) upon the binomial basis is 9, with a standard deviation of 3·32, or a difference equal to 2·56 times its standard error and giving only a probability of the difference being due to chance of 5 in one thousand.

That a decided bias, in error, would be brought about by not dropping the dead birds from consideration is evident. Study of the records of the dead birds of both pens indicated that while mortality was approximately the same (17·6 per cent for pen A and 14·2 per cent for pen B), a large percentage of the birds of the latter pen remained alive for long periods in too poor condition to produce eggs, whereas those of pen B, once out of laying condition, succumbed fairly rapidly. Apart from this consideration, it is but logical to presume that, since those birds which were in poor condition and not laying for long periods were definitely far outside the normal distribution for their pen, for reasons which, as shown by postmortems, could not possibly be attributable to the treatment which the pen was receiving, their lack of production should not be charged against the mean of the pen. It would appear from these data and from previous observation, that birds which die are very likely to be abnormal for unknown periods prior to death and hence their records should be completely removed from the data. This would not be possible except where birds are caged singly and individual feed consumption figures are available.

It will be noted from Table 5 (Appendix A) that a lower variability of production was the case for pen B as compared to that of pen A during twenty-five weeks out of the forty-four. Analysis by the binomial method indicates this difference to be in no way significant.

(2) Total Weight Production of Eggs per Bird, body weight to maintain, gain or loss in body weight and efficiency of use of feed, on a weekly basis for the test period (44 weeks).—Tables 6 and 7 (Appendix A) show the complete details of initial weight, final weight, production, body weight maintenance and gain or loss in weight for pens A and B, respectively, for each bird which lived through the test.

Table 2, following, summarizes the data of tables 6 and 7 (Appendix A).

TABLE 2.—DIFFERENCES IN AVERAGE INITIAL WEIGHT, FINAL WEIGHT, GAIN, BODY WEIGHT TO MAINTAIN, FEED CONSUMPTION AND TOTAL WEIGHT OF EGGS FOR BOTH PENS (WEEKLY AVERAGES PER BIRD).

Criterion	Lot having greater weight	Difference	P†
		grams	
Initial weight	A	10·6±35·2*	·76
Final weight	A	3·2±56·3	·95
Gain	B	0·1± 1·0	·86
Body weight to maintain	A	6·4±48·9	·86
Feed consumption	A	6·8±16·4	·68
Total weight of eggs laid	A	0·8± 6·8	·90

*Standard error.
†P = ·05, or less, taken as necessary level of significance.

The feed consumed is the average weekly consumption per bird; the body weight to maintain is the average of weekly body weights for the whole experimental period (44 weeks); the production is the average production of egg material per bird per week; the gain is the average gain per bird per week.

Obviously there is no suggestion of significance in any differences occurring between these lots in any of the above criteria.

In a trial of this kind, the best measure of the effect of the imposed experimental treatment is the relative efficiency with which the rations under comparison are utilized for the several body functions. In this instance, these include the requirements for maintenance of body weight, gain or loss in weight and eggs produced. An ideal condition would be one where all these factors were alike for both lots, in which case differences in actual feed consumption would be directly comparable and would be caused by the effect of the experimental treatment, and/or any uncontrolled factors which cannot be identified or are unmeasurable and which constitute what is termed "experimental error," by the magnitude of which the significance of observed differences, with respect to the experimental treatment, is judged.

It is obviously impossible to control variations in body weight, gain or loss in weight, or egg production between birds, and hence their effect on variations in feed intake and utilization. It is possible, however, by the use of the method of partial regression as described by Crampton and Hopkins (1934) (1934a) to adjust the observed feed consumptions for the effects of the correlated variables (body weight to maintain, gain or loss in weight and weight of eggs produced), thus permitting a truer estimate of "experimental error." The details of this statistical treatment are given in Appendix B.

The results of the analysis may be summarized as follows:—

Adjusted mean feed consumption—			
Lot A (unprocessed oil)	Lot B (deaminized oil)	Difference in favour of lot	P
809.5 grams	797.5 grams	B: 12.0±9.35*	.20

*Standard error.

A study of the above data indicates that a difference as great or greater than that actually occurring (12·0 grams) would occur by chance only once in five trials. This difference is, therefore, below the generally accepted probability for biological work of this nature of once in twenty trials ($P = .05$). It is understood, however, that no hard and fast rule can be set for significance for all trials and that the level of significance must depend, within reasonable limits, upon the nature of the actual data under consideration. In this instance, the fact that a well marked general trend in favour of this lot during the entire period of growth existed and that certain uncontrollable circumstances, later to be discussed, definitely worked against this lot, must be taken into consideration. Under the circumstances the above difference may be safely considered to have some importance in a consideration of the efficiency of the treatment under experiment.

It may be concluded, then, that the removal of the nitrogenous fraction from the cod liver oil permitted pen B to make more efficient use of its feed than would otherwise have been the case.

III. DISCUSSION

The experimental evidence here presented will be discussed from the standpoint of growth, production and mortality, with particular reference to circumstances not mathematically measurable but which should be taken into consideration in arriving at an appraisal of the net effect of the treatments accorded to the two lots.

A. Growth

In spite of the fact that by chance lot A was heavier at time of hatch, lot B overcame this disadvantage after the fourth week. When the birds were separated according to sex it was found that the males receiving the deaminized oil were heavier throughout, with the exception of at hatching time, the difference increasing steadily from the fifth to the eighth week. The females of lot B, however, while heavier throughout, with the exception of the first two weeks, were not significantly so. There was, however, a significant superiority in uniformity for the whole period for the females receiving the deaminized oil, as indicated by the binomial test.

It may be judged then, that deaminization of the cod liver oil was responsible for superior uniformity and growth during the first eight weeks of the growing period and that this superiority was particularly marked with the males.

During the rearing period, which included females only, or from nine weeks of age to maturity, lot B was still superior in body weight in every case excepting in the twenty-fourth week. The difference in uniformity, in fact, was in favour of the females receiving the deaminized oil from hatch to maturity. It is evident therefore that deaminization of this oil increased the uniformity of this lot over their control to a highly significant degree. While uniformity has not in the past been particularly stressed, it is in the author's opinion based on observation of individuals on normal and abnormal treatments in experimental trials, a factor of great importance which should be given a high rating as a criterion of the efficiency of a feed or treatment.

The first evident difference in favour of the lot receiving the unprocessed oil was in their earlier sexual maturity and the attainment of a greater body weight at maturity. While early maturity is considered to be a desirable characteristic, it is not possible to state whether early maturity in comparable lots is an expression of a good nutritional condition, or otherwise. The possibility of a larger proportion of genetically early maturing birds occurring in the one pen cannot be overlooked, although such a condition is unlikely in view of the precautions of allotment. This factor of early maturity, therefore, obviously cannot be definitely interpreted.

A study of the data indicates no explanation of the greater weight of the birds in pen A at maturity. The difference actually was slight, with a probability of occurrence, by chance alone, of seventy-six times in one hundred. It should be noted also that this mature weight difference did not persist through the period of egg production.

B. Production

As previously pointed out, efficiency of performance in egg production cannot rest solely on the quantity of egg material produced but on that plus the body weight which had to be maintained and the gain or loss in body weight, all three of which must in turn be related to the amount of feed required to support these functions.

In considering the actual total weight of eggs produced, the difference existing, which is in favour of the pen receiving the unprocessed oil, is insignificant, being actually $0{\cdot}8 \pm 6{\cdot}8$ grams per bird per week, a difference which might occur by chance alone 90 times out of 100. Similarly, initial weight, final weight, body weight and feed consumption were all slightly greater for pen A but with probabilities of these differences occurring by chance alone of 76 per cent, 95 per cent, 86 per cent and 68 per cent, respectively. Body weight gain alone was larger for the processed oil pen with a probability of chance occurrence of 86 times in 100.

It is apparent that, individually, no difference exists in any of the factors which go to make up the production complex. The efficiency of use of feed, however, is a single measure which combines the effects of maintenance and production. After adjustment, by the method of partial regression, to account for the effect upon feed requirement of differences in body weight to maintain, production and gain in body weight, a difference in feed requirement was found in favour of the birds receiving the deaminized oil, of 12 grams per bird per week or 1·5 per cent. Statistical analysis indicated that such a difference would be expected to occur by chance alone only once in five trials.

In further considering the matter of production several additional facts should be noted. As previously mentioned, the lot receiving the unprocessed oil reached sexual maturity at an earlier period than did the other lot. When the first eggs were laid by the lot receiving the unprocessed oil, records of the egg production period for both lots started immediately. Consequently there was a period of from ten days to two weeks during which production was much greater for that lot (lot A) and also during which the other lot was at a decided disadvantage because of the fact that these pullets were gaining body weight rapidly (as indicated by chart 1) with a consequent very heavy feed requirement, but were producing very little product in eggs. At the end of the test, this lot (lot B) was again at an unavoidable disadvantage as they were laying at a greater rate at that time as indicated by Table 5 (Appendix A). Actually, twenty-four pullets were still laying in the lot receiving deaminized oil whereas only nineteen were in production for the other lot at the end of the test. There is little doubt that had production been permitted to continue to the end of the production period the data would have been even more favourable for those receiving the processed oil.

C. Mortality

Since the effect of the treatment which either one of these lots received was not sufficiently marked to seriously alter the processes of growth or egg production, it would not be expected that differences in mortality would be particularly evidenced. This proved to be the case. During the brooding period, a mortality of 7·0 per cent was experienced by the pen on the unprocessed oil as against 5·4 per cent for the deaminized oil pen. For the rearing and egg production periods, the mortality was 20·9 per cent and 19·1 per cent, and 17·6 per cent and 14·2 per cent for the two pens, respectively. While the mortality was actually higher in all cases for the pen receiving the unprocessed oil it is probable that no significance can be attached to this fact.

IV. SUMMARY AND CONCLUSIONS

A survey of the literature has indicated that cod liver oil has, in many instances, contained toxic properties which have been shown to be decidedly detrimental to animals and poultry. Among these, certain amine compounds have been shown to be extremely toxic. The level of their occurrence in certain cod liver oils of different types was determined. A technique was established for the removal from cod liver oils of these end products of protein decomposition.

A sample of cod liver oil, typical of those oils in which liver material might exist and of a type which is commonly fed to poultry in many quarters, was subjected to this process and fed, in comparison with an unprocessed portion of the same oil, to comparable lots of chickens from time of hatch to the end of the first laying year at levels commonly used in poultry rations. The following deductions are made from a consideration of the data obtained.

(1) All fish oils tested contained measurable quantities of nitrogenous materials of possible toxic effect ranging from 0·0003 to 0·031 per cent of the oil. Carefully refined oils for human consumption contained an appreciable quantity of these products. Pilchard oil contained a much smaller proportion of these impurities than did cod liver oils, with the exception only of the refined medicinal oils. Certain sun-rendered oils were relatively high in these impurities.

(2) While oils of high free fatty acid content are not necessarily also high in these impurities, they do generally go hand in hand. It might be said that on the average the condition of the original livers when extracted, the care and methods of extraction, or the conditions of storage, or a combination of these factors, determine the level of both of these fractions in the oil although they are not themselves interrelated. A high free fatty, acid oil is therefore undesirable for this reason.

(3) The removal of these impurities (nitrogenous fraction) by chemical processing permitted of greater growth in young chicks throughout the growth period. The retarding effect of these impurities was particularly marked with the cockerels, whereas the pullets were less severely affected. Uniformity of growth was definitely improved in the females throughout the entire growth period, when these impurities were removed from the oil.

(4) Efficiency of use of feed for purposes of egg production, maintenance of body weight and gain in body weight, during the first laying year, was slightly increased by the removal of this fraction of the oil.

(5) The mortality was affected to a limited extent only, if at all, by this treatment of the oil.

(6) Since a definite toxic effect of the nitrogenous fraction of cod liver oils on growth has been demonstrated, with a strong suggestion of the existence of a similar effect on the efficiency of use of feed during the period of egg production,

it is concluded that oils produced under such conditions that this fraction may be high, should not be used for poultry-feeding purposes. Since this fraction is nitrogenous in nature it is, therefore, foreign to a pure oil and must arise from contamination of liver material during some stage of production of the oil.

ACKNOWLEDGMENTS

The helpful assistance of the following is gratefully acknowledged:—

Mr. C. H. Robinson, Acting Dominion Chemist, and members of his staff for all chemical analyses and for the processing of the large quantity of oil used in these tests, which amounted to no inconsiderable task; to Mr. Robinson in particular, for advice concerning the chief chemical features of the impurities of cod liver oils and for his continuous interest in the project.

Dr. R. H. F. Manske, of the Department of Chemistry of the National Research Council, for a method of removing the nitrogenous impurities of cod liver oil.

Prof. E. W. Crampton, of the Department of Animal Husbandry of Macdonald College (McGill University), for his assistance in the determination of suitable methods for the statistical analysis of the data presented and for many helpful suggestions in the interpretation of data.

REFERENCES

Agduhr, E.
1926. Post-natal development under different conditions of nutrition and circumstances of functioning. Acta Paediatrica, 5: 319.
1927. Changes in the organism caused by cod liver oil added to the food. Acta Paediatrica, 6: 165.
1928. Possible danger of cod liver oil. British Med. J. 1: 639.

Bell, M. E., *et al*
1933. Studies of the alleged toxic action of cod liver oil and concentrates of vitamin A. Ztschr. f. vitaminforschung 2: 163—Cited Nut. Abstr. Rev. 3: 689.

Bird, S., and H. S. Gutteridge
1934. Variation of sex difference in chick growth. Scientific Agric. 14: 8, 433.
1934a. Significance determination numbers. Scientific Agric. 14: 10, 547.

Buckner, G. D., *et al*
1934. Growth of White Leghorn Chicks. Poultry Sc. 13: 2, 110.

Crampton, E. W., and J. W. Hopkins
1934. The use of the method of partial regression in the analysis of comparative feeding trial data. J. Nut. 8: 3, 113.
1934a. Part 2 J. Nut. 8: 3, 329.

Fraps, G. S.
1928. Digestibility and production coefficients of poultry feeds. Texas Agric. Exp. Station Bul. 372.

Gautier, A., and L. Mourgues
1888. Sur. les alkaloids de l'huile de foie de morue. Compt. Rend. Acad. Sc. 107: 110, 254, 626, 740.

Gutteridge, H. S.
1931. Unpublished data.
1931a. Vitamin A and D studies with growing chicks. Scientific Agric. 12: 6, 327.
1932. Unpublished data.
1932a. Vitamin A and D studies with growing chicks: A comparison of cod liver oil and pilchard oil as sources of the growth-promoting factor, Vitamin A. Scientific Agric. 13: 6, 374.

Harris, L. J., and T. Moore
1928. Hypervitaminosis and vitamin balance. Biochem. J. 22: 1461.

Hawk, P. B.
1908. On the leucomaines of cod liver oil. Am. Jour. Physiol. 21: 22.

Hawk, P. B., and O. Bergeim
1927. Practical Physiological Chemistry, page 770, 9th edition Blakiston's Son & Co. Philadelphia.

Holmes, A. D., *et al*
1930. Does cod liver oil of high acid content have toxic properties? Poultry Sc. 9: 3, 164.

Hunter, J. E., *et al*
1931. The response of the growing chick to oils of varying acidity. Penn. Agric. Exp. Station Bul. 266, p. 7.

Miles, S. R.
1935. A very rapid and easy method of testing the reliability of an average and a discussion of the normal and binominal methods. J. Am. Soc. Agron. 27: 1, 21.

Miller, R. J., *et al*
1929. Nutritional leg weakness in poultry. Poultry Sc. 8: 3, 113.

Mitchell, H. H., and H. S. Grindley
1913. The element of uncertainty in the interpreting of feeding experiments. Illinois Univ. Exp. Station, Bul. 165.

Norris, E. R., and A. E. Church
1930. The toxic effect of fish liver oils and the action of vitamin B. J. Biol. Chem. 89: 1, 437.

Slagswold, J. J.
1925. Tranforgiftninger hos kalver. Norsk. Veterinaer Tideskrift 6: 161. Cited J.A.V.M.A. 18: 236.

Warren, D. C.
1930. Crossbred poultry. Agric. Exp. Sta., Kansas State Agric. College. Bul. 252.

Wynter Blyth, A., and M. Wynter Blyth
1920. Poisons: their effect and detection. Charles Griffin & Co., Ltd., London.

Yamamoto, J.
1934. Toxicity of fish liver oils and fish oils and antitoxic effect of yeast. Bul. Inst. Phys. Chem. Res., .(Japan) 13: 1.

APPENDIX A

ORIGINAL DATA FOR THE BROODING, REARING AND EGG PRODUCTION PERIODS, WITH NECESSARY STATISTICAL CONSTANTS

Appendix A—Table 1

MEAN BODY WEIGHT, VARIABILITY, MEAN DIFFERENCES AND SIGNIFICANCE OF DIFFERENCES FOR MALES AND FEMALES COMBINED (1 TO 8 WEEKS)

Age	Pen A—untreated oil			Pen B—deaminized oil			Difference in means	S.E. of difference	In favour of	P
	Number of birds	Body weight		Number of birds	Body weight					
		Mean	C.V.		Mean	C.V.				
Hatch		gms.	gms.		gms.	gms.				
.....	284	35·68	10·21	279	34·21	9·29	1·47	0·288	A	0·00
1st week.....	278	55·81	13·84	272	54·65	13·89	1·16	0·653	A	0·07
2nd week.....	273	90·37	14·94	271	89·84	14·93	0·53	1·154	A	0·64
3rd week.....	271	136·66	15·82	270	139·26	14·90	2·60	1·823	B	0·15
4th week.....	271	203·14	16·10	270	206·00	15·81	2·86	2·807	B	0·31
5th week.....	267†	287·76	13·89	*267	294·86	14·70	7·10	3·606	B	0·05
6th week.....	*264	379·02	13·87	*263	388·18	13·70	9·16	4·607	B	0·05
7th week.....	264	481·74	12·83	*263	497·68	13·21	15·94	5·564	B	0·00
8th week.....	*263	589·40	12·53	*261	608·46	12·91	19·06	6·664	B	0·00

*One chick significantly outside distribution.
†Two chicks selected at random removed for other work.

Appendix A—Table 2

MEAN BODY WEIGHT, VARIABILITY, MEAN DIFFERENCES AND SIGNIFICANCE OF DIFFERENCES FOR MALES AND FEMALES SEPARATELY (HATCH TO EIGHT WEEKS).

Age	Pen A—untreated oil			Pen B—deaminized oil			Difference in means	S.E. of difference	In favour of	P.
	Number of birds	Males		Number of birds	Males					
		Body weight			Body weight					
		Mean	C.V.		Mean	C.V.				
		gms.	gms.		gms.	gms.				
Hatch.....	90	37·04	9·92	88	36·93	8·58	0·11	0·51	A	0·83
1st week.....	90	56·93	11·89	88	59·32	12·35	2·39	1·06	B	0·02
2nd week.....	90	89·80	15·76	88	90·18	16·70	0·38	2·19	B	0·86
3rd week.....	90	135·88	15·36	88	143·18	16·15	7·30	3·30	B	0·03
4th week.....	90	202·00	15·33	88	208·18	16·62	6·18	4·92	B	0·21
5th week.....	90	290·00	13·87	88	300·00	14·91	10·00	6·38	B	0·12
6th week.....	90	379·99	15·27	88	395·22	15·23	15·23	8·86	B	0·08
7th week.....	90	489·76	14·18	88	509·08	14·78	19·32	10·86	B	0·07
8th week.....	90	602·24	13·18	88	630·00	14·14	27·76	12·65	B	0·03
		Females			Females					
Hatch.....	90	35·58	8·69	88	35·48	8·15	0·10	0·45	A	0·98
1st week.....	90	56·27	13·41	88	54·41	11·03	1·86	1·02	A	0·07
2nd week.....	90	92·49	14·06	88	89·72	11·63	3·76	1·76	A	0·03
3rd week.....	90	139·22	15·29	88	141·82	12·19	2·60	2·90	B	0·37
4th week.....	90	209·34	14·63	88	205·90	11·45	3·44	4·09	A	0·40
5th week.....	90	290·00	12·26	88	292·28	12·14	2·28	5·46	B	0·67
6th week.....	90	381·34	12·63	88	385·00	10·77	3·66	6·78	B	0·59
7th week.....	90	481·34	10·89	88	485·46	10·41	4·12	7·71	B	0·60
8th week.....	90	580·88	10·83	88	588·86	9·50	7·98	8·81	B	0·36

Appendix A—Table 3

MEAN BODY WEIGHT, VARIABILITY, MEAN DIFFERENCES AND SIGNIFICANCE OF DIFFERENCES FOR FEMALES ONLY (9 TO 24 WEEKS INCLUSIVE)

Age	Pen A—untreated oil			Pen B—deaminized oil			Difference in means	S.E. of difference	In favour of	P.
	Number of birds	Body weight		Number of birds	Body weight					
		Mean	C.V.		Mean	C.V.				
weeks		gms.	gms.		gms.	gms.				
9th	89	625·55	11·73	89	633·98	10·65	8·43	10·59	B	0·42
10th	88	727·28	12·29	88	736·84	10·08	9·56	12·43	B	0·44
11th	86	790·70	15·35	89	825·28	11·03	34·58	16·40	B	0·04
13th*	85	978·24	14·84	87	1,001·72	12·10	23·48	20·54	B	0·25
14th	82	1,070·70	13·59	84	1,092·90	11·58	22·20	21·31	B	0·29
15th	82	1,189·00	13·25	84	1,202·40	11·07	13·40	22·80	B	0·55
16th	84	1,273·80	11·72	80	1,285·00	9·46	11·20	21·29	B	0·61
17th	81	1,345·10	10·57	76	1,363·16	7·92	18·10	20·21	B	0·37
18th	82	1,406·10	10·77	76	1,426·06	8·63	20·00	22·03	B	0·36
19th	81	1,448·77	10·09	76	1,468·65	8·59	19·88	21·82	B	0·36
20th	79	1,489·24	9·93	76	1,503·95	8·73	14·71	22·58	B	0·51
21st	76	1,560·53	10·22	74	1,539·19	8·71	21·34	24·19	A	0·39
22nd	75	1,579·30	10·30	74	1,591·89	8·90	12·59	25·25	B	0·62
23rd	75	1,618·00	11·60	72	1,649·70	9·90	31·70	29·31	B	0·28
24th	72	1,880·56	11·59	72	1,855·81	9·09	21·75	32·71	A	0·45

*Chicks not weighed at expiration of 12th week owing to slight infection of bronchitis.

Appendix A—Table 4

PERCENTAGE VARIABILITY IN GROWTH FOR FEMALES ONLY (HATCH TO 24 WEEKS)

Age	Pen A—untreated oil		Pen B—deaminized oil		Difference in C.V.'s	S.E. of difference	In favour of	P
	Number of birds	Coefficient of variability	Number of birds	Coefficient of variability				
Hatch	90	8·69	88	8·15	0·54	0·899	B	0·55
1 week	90	13·41	88	11·03	2·38	1·319	B	0·07
2 weeks	90	14·06	88	11·63	2·43	1·389	B	0·08
3 weeks	90	15·29	88	12·19	3·10	1·492	B	0·03
4 weeks	90	14·63	88	11·45	3·18	1·415	B	0·02
5 weeks	90	12·26	88	12·14	0·12	1·274	B	0·92
6 weeks	90	12·63	88	10·77	1·86	1·260	B	0·14
7 weeks	90	10·89	88	10·41	0·48	1·141	B	0·67
8 weeks	90	10·83	88	9·50	1·33	1·178	B	0·26
9 weeks	89	11·73	89	10·65	1·08	1·220	B	0·37
10 weeks	88	12·29	88	10·08	2·21	1·227	B	0·07
11 weeks	86	15·35	89	11·03	4·32	1·458	B	0·00
13 weeks*	85	14·84	87	12·10	2·74	1·507	B	0·07
14 weeks	82	13·59	84	11·58	2·01	1·428	B	0·16
15 weeks	82	13·25	84	11·07	2·18	1·391	B	0·12
16 weeks	84	11·72	80	9·46	2·26	1·212	B	0·06
17 weeks	81	10·57	76	7·92	2·65	1·079	B	0·01
18 weeks	82	10·77	76	8·63	2·14	1·123	B	0·06
19 weeks	81	10·09	76	8·59	1·50	1·074	B	0·16
20 weeks	79	9·93	76	8·73	1·20	1·087	B	0·24
21 weeks	76	10·22	74	8·71	1·51	1·123	B	0·18
22 weeks	75	10·30	74	8·90	1·40	1·143	B	0·22
23 weeks	75	11·60	72	9·90	1·70	1·294	B	0·19
24 weeks	72	11·59	72	9·09	2·50	1·264	B	0·05

*Chicks not weighed at expiration of 12th week owing to infectious bronchitis

Appendix A—Table 5

TOTAL WEIGHT PRODUCTION OF EGGS PER BIRD, WEEKLY AVERAGES

Age	Pen A—untreated oil			Pen B—deaminized oil			Difference in means	S.E. of difference	In favour of
	Number of birds	Weight of production		Number of birds	Weight of production				
		Mean	C.V.		Mean	C.V.			
weeks		gms.	gms.		gms.	gms.			
24	53	16·42	133·98	54	25·92	158·17	9·50	6·42	B
25	53	35·25	154·89	54	56·10	150·26	20·85	13·82	B
26	53	138·39	70·23	54	157·20	60·68	18·81	18·76	B
27	53	245·37	40·10	54	244·99	35·38	0·38	18·11	A
28	53	288·96	18·15	54	269·43	24·16	19·53	11·53	A
29	53	275·94	16·53	54	271·11	20·80	4·83	10·00	A
30	53	249·90	27·47	54	241·11	30·48	8·79	13·89	A
31	53	270·84	22·48	54	268·32	21·24	2·52	11·53	A
32	53	276·51	24·19	54	280·56	17·21	4·05	11·40	B
33	53	281·55	26·95	54	271·11	18·03	10·44	12·57	A
34	53	277·50	22·27	54	292·77	16·39	15·27	10·86	B
35	53	284·43	20·56	54	301·65	13·52	17·22	9·85	B
36	53	274·83	26·30	54	263·31	35·55	11·52	16·25	A
37	53	291·24	24·61	54	278·34	27·16	12·90	14·38	A
38	53	303·12	18·90	54	313·32	14·84	10·20	10·20	B
39	53	325·74	18·69	54	301·11	22·11	24·63	12·45	A
40	53	320·07	13·87	54	301·11	20·92	18·96	10·63	A
41	53	287·25	33·31	54	291·66	27·25	4·41	17·18	B
42	53	307·08	17·87	54	306·66	19·46	0·42	11·18	A
43	53	312·75	22·06	54	302·22	18·86	10·53	12·37	A
44	53	299·16	23·86	54	315·54	14·07	16·38	11·75	B
45	53	293·49	24·02	54	307·23	18·42	13·74	12·48	B
46	53	276·51	24·73	54	292·77	21·41	16·26	12·81	B
47	53	261·78	32·31	54	267·75	29·80	5·97	16·06	B
48	53	264·63	25·05	54	256·11	27·87	8·52	13·45	A
49	53	262·35	30·55	54	243·90	37·26	18·48	17·09	A
50	53	262·35	31·10	54	253·32	32·80	9·03	16·06	A
51	53	258·96	34·87	54	237·75	36·59	21·21	17·32	A
52	53	240·21	44·07	54	246·09	38·64	5·88	19·65	B
53	53	241·41	42·25	54	237·21	39·07	4·20	19·00	A
54	53	256·14	37·59	54	258·90	26·07	2·76	16·25	B
55	53	227·25	45·41	54	242·76	29·90	15·51	17·43	B
56	53	215·94	42·92	54	247·20	30·94	31·26	16·61	B
57	53	226·11	47·30	54	238·86	34·03	12·75	18·57	B
58	53	222·18	44·96	54	249·90	27·50	27·72	16·76	B
59	53	228·39	42·69	54	234·95	27·58	6·56	16·19	B
60	53	221·61	39·52	54	220·53	39·85	1·08	17·12	A
61	53	218·79	42·23	54	188·88	47·83	29·91	17·75	A
62	53	201·78	49·50	54	186·66	56·89	15·12	20·10	A
63	53	153·66	66·38	54	144·99	67·86	8·67	19·54	A
64	53	142·35	73·97	54	115·56	95·53	26·79	21·05	A
65	53	125·94	84·80	54	112·77	93·64	13·17	20·74	A
66	53	115·30	98·00	54	102·24	94·48	13·06	20·35	A
67	53	70·47	122·60	54	78·87	109·93	8·40	16·88	B

Appendix A—Table 6

INITIAL WEIGHT, FINAL WEIGHT, GAIN OR LOSS IN BODY WEIGHT, BODY WEIGHT TO MAINTAIN, FEED CONSUMPTION, WEIGHT OF EGGS, FOR THE PERIOD OF EGG PRODUCTION (WEEKLY AVERAGES).

PEN A

Bird number	Initial weight	Final weight	Gain or loss	Average body weight to maintain	Average feed consumption	Average total weight of eggs laid
	gms.	gms.	gms.	gms.	gms.	gms.
1016	1,464	1,901	9·9	1,679	768	248·8
1022	2,121	2,040	− 1·8	2,181	819	272·3
1024	1,495	1,352	− 3·2	1,541	677	198·8
1026	1,904	1,932	0·6	2,018	694	248·5
1028	1,973	2,115	3·2	2,376	1,043	255·3
1034	2,007	2,216	4·7	2,218	781	251·1
1036	1,855	2,240	8·7	2,153	850	243·1
1038	2,234	2,251	0·4	2,428	797	165·2
1040	1,942	2,254	7·1	2,500	972	224·3
1044	2,368	2,316	− 1·2	2,720	809	222·9
1048	1,917	1,676	− 5·5	1,938	899	282·9
1050	2,033	1,919	− 2·6	2,164	765	186·8
1052	1,579	1,925	7·9	1,999	728	185·7
1054	1,655	2,100	10·1	2,066	866	267·9
1056	1,744	1,634	− 2·5	1,929	728	199·4
1060	1,657	2,203	12·4	2,038	872	272·8
1062	1,874	2,279	9·2	2,373	917	242·8
1064	1,727	2,040	7·1	1,985	761	247·3
1066	1,917	2,028	2·5	2,141	806	242·3
1068	1,812	1,699	− 2·6	2,237	797	225·7
1070	1,815	1,817	0·1	1,848	669	246·0
1072	1,829	2,014	4·2	2,070	914	294·1
1074	2,397	2,454	1·3	2,790	921	244·5
1078	1,857	1,535	− 7·3	1,838	749	266·2
1080	1,960	2,421	10·5	2,399	916	323·4
1082	1,905	2,168	6·0	2,227	895	141·5
1084	1,832	2,217	8·7	2,170	845	260·6
1086	1,882	2,367	11·0	2,370	902	258·5
1088	1,945	2,305	8·2	2,260	907	246·9
1092	1,734	1,676	− 1·3	2,028	777	215·0
1100	2,020	2,595	13·1	2,397	907	279·1
1102	1,881	2,119	5·4	2,075	896	259·8
1104	1,735	1,813	1·7	2,084	799	261·8
1108	2,000	2,162	3·7	2,433	934	271·6
1110	1,634	2,049	9·4	1,837	811	277·6
1114	1,913	2,156	5·5	1,902	819	239·6
1120	1,747	1,787	0·9	2,196	638	150·1
1122	1,472	1,411	− 1·4	1,406	676	232·1
1124	1,953	1,953	0·0	2,021	746	231·1
1128	1,654	1,665	0·2	1,841	774	208·7
1130	1,982	1,911	− 1·6	2,119	798	185·1
1132	1,947	1,938	− 0·2	2,148	836	249·7
1134	1,591	1,424	− 3·8	1,712	614	203·6
1136	1,906	1,925	0·4	1,794	771	236·2
1138	1,966	2,127	3·7	2,370	862	271·3
1140	1,823	1,915	2·1	2,116	766	149·7
1142	2,057	1,833	− 5·1	2,005	687	234·5
1144	1,631	1,689	1·3	1,981	818	230·2
1146	1,833	1,679	− 3·5	1,942	665	207·2
1148	2,020	1,927	− 2·1	1,960	753	211·1
1152	2,007	1,877	− 2·9	2,197	743	161·0
1154	1,475	1,653	4·0	1,744	753	209·0
1156	1,834	1,883	1·1	2,003	855	212·0
*	.....	.....	2·6	2,093	807	233·5
Sum	98,515	104,585	140·3	113,060	43,572	12,586·2
Mean	1,858·8±27·5†	1,973·3±38·0	2·60± 0·69	2,093·7±35·7	806·9±12·2	233·1±5·2
Standard deviation	200·2±19·4	276·5 ±26·8	5·05±0·49	262·7±25·3	89·5±8·61	38·2± 3·68
Coefficient of variability	10·72± 1·05	14·01± 1·39	194·2±55·68	12·55± 1·23	11·09± 1·04	16·39± 1·62

*Calculated.
†Standard errors throughout.

Appendix A—Table 7

INITIAL WEIGHT, FINAL WEIGHT, GAIN OR LOSS IN BODY WEIGHT, BODY WEIGHT TO MAINTAIN, FEED CONSUMPTION, WEIGHT OF EGGS, FOR THE PERIOD OF EGG PRODUCTION (WEEKLY AVERAGES).

PEN B

Bird number	Initial weight	Final weight	Gain or loss	Average body weight to maintain	Average feed consumption	Average total weight of eggs laid
	gms.	gms.	gms.	gms.	gms.	gms.
1017	1,850	1,940	2·0	2,149	763	184·0
1019	2,116	2,067	− 1·1	2,090	798	242·4
1021	1,800	2,082	6·4	1,983	724	246·6
1023	1,820	2,017	4·5	2,154	797	202·8
1025	2,117	2,363	5·6	2,663	955	199·9
1027	1,802	1,817	0·3	1,800	975	258·7
1031	1,658	1,740	1·8	2,055	702	220·2
1035	1,967	2,362	9·0	2,244	888	281·4
1039	2,116	2,566	10·2	2,719	968	272·2
1043	1,945	1,997	1·2	2,316	821	212·7
1045	1,746	1,721	− 0·6	2,033	782	219·6
1047	1,922	2,216	6·7	2,281	864	272·1
1049	1,656	1,865	4·7	1,819	839	277·0
1055	2,166	2,947	17·7	2,694	877	209·3
1059	2,017	1,803	− 4·9	2,328	774	263·7
1063	2,072	2,020	− 1·2	2,444	747	196·5
1065	1,767	2,013	5·6	2,274	859	211·3
1067	1,983	2,165	4·1	2,073	858	258·3
1069	2,060	2,362	6·9	2,384	893	267·1
1071	1,642	1,861	5·0	1,991	740	213·2
1075	1,820	1,970	3·4	2,312	753	204·1
1077	1,833	2,040	4·7	2,202	832	237·6
1079	2,134	2,015	− 2·7	2,362	765	189·5
1081	1,940	2,102	3·7	2,097	718	242·5
1085	1,905	1,695	4·8	1,783	780	201·7
1087	1,603	1,690	2·0	1,709	621	170·4
1089	1,787	1,690	− 2·2	2,051	864	263·7
1093	1,573	1,898	7·4	1,816	737	251·0
1095	1,624	1,275	− 7·9	1,787	723	210·8
1097	1,700	1,562	− 3·1	1,953	868	232·1
1099	1,747	1,470	− 6·3	1,847	918	254·7
1101	2,066	2,171	2·4	2,375	896	236·2
1103	1,856	1,812	− 1·0	1,760	704	232·4
1105	1,830	2,140	7·0	2,159	899	267·1
1107	1,770	1,973	4·6	1,782	789	261·0
1109	1,702	1,773	1·6	1,819	717	279·0
1111	1,594	1,648	1·2	1,616	684	266·7
1115	1,709	1,557	− 3·4	1,859	681	191·0
1117	1,660	1,813	3·5	1,778	753	227·5
1121	1,913	2,341	9·7	2,268	848	277·5
1123	1,896	2,386	11·1	2,141	767	167·8
1125	1,887	1,960	1·7	2,052	735	219·9
1127	1,850	1,491	− 8·2	1,832	636	167·6
1131	1,819	1,624	− 4·4	1,785	798	216·5
1133	1,853	1,810	− 1·0	2,122	859	200·0
1135	1,792	1,703	− 2·0	2,181	746	248·4
1137	1,978	2,236	5·9	2,318	869	226·5
1139	1,683	2,249	12·9	2,122	799	218·1
1141	1,920	1,800	− 2·7	1,971	815	274·6
1145	2,129	2,495	8·3	2,324	834	265·0
1147	1,517	1,922	9·2	1,886	707	222·5
1151	1,810	2,179	8·4	2,160	830	189·3
1153	1,878	2,046	3·8	1,937	868	265·8
1157	1,803	1,923	2·7	1,944	771	257·0
Sum	99,803	106,383	149·4	112,604	43,208	12,544·5
Mean	1,848·2±22·0	1,970·1±41·5	2·77± 0·73	2,085·3±34·8	800·1±11·0	232·2±4·4
Standard deviation	162·0±15·6	305·4±29·4	5·36± 0·52	255·7±24·6	81·0± 7·79	32·2± 3·10
Coefficient of variability	8·77± 0·85	15·50±1·53	193·5±54·92	12·26± 1·23	10·12± 0·98	13·86± 1·36

APPENDIX B

STATISTICAL ANALYSIS OF DATA FOR THE PERIOD OF EGG PRODUCTION

Appendix B

The analysis of variance for the egg production period from which the estimate of "error" has been obtained is shown in table 1 (Appendix B).

The material presented in this table has been calculated directly from the data of tables 6 and 7 (Appendix A). From these data partial regression coefficients were obtained by substitution in the following simultaneous equation:—

$$b_1S(x_1^2)+b_2S(x_1x_2)+b_3S(x_1x_3) = S(yx_1)$$
$$b_1S(x_1x_2)+b_2S(x_2^2)+b_3S(x_2x_3) = S(yx_2)$$
$$b_1S(x_1x_2)+b_2S(x_2x_3)+b_3S(x_3)^2 = S(yx_3)$$

where y is the dependent variable (feed) and x_1, x_2 and x_3 are respectively body weight to maintain, weight production of eggs and gain in body weight, and b_1, b_2 and b_3 the respective partial regressions of body weight to maintain, weight production of eggs and gain in body weight upon feed consumption.

Using the data of the error line of table 1 (Appendix B) the solution of this equation gives:

$$b_1 = -0{\cdot}01548$$
$$b_2 = -0{\cdot}04379$$
$$b_3 = 27{\cdot}87415$$

From these regressions the sum of squares of feed intake may be corrected for the effects of variation in body weight, weight production and gain in weight by virtue of the following relationship:—

Corrected sums of squares of feed, $S(y^2) = S(y-b_1x_1-b_2x_2-b_3x_3)$

This square for the line from which the regressions were calculated reduces to $S(y^2)-b_1S(yx_1)-b_2S(yx_2)-b_3S(yx_3)$ and substitution yields the corrected sum of squares for feed consumption shown in table 2 (Appendix B).

Appendix B—Table 1

SUMS OF SQUARES AND PRODUCTS FOR ANALYSIS OF VARIANCE AND COVARIANCE OF FEED CONSUMPTION (y), BODY WEIGHT TO MAINTAIN (x_1), WEIGHT PRODUCTION OF EGGS (x_2), AND GAIN IN BODY WEIGHT (x_3).

Due to:	D/F	Sum of squares				Sum of products					
		$S(y^2)$* (Feed)	$S(x_1^2)$ (Body wt)	$S(x_2^2)$ (Prod.)	$S(x_3^2)$ (Gain)	$S(yx_1)$†	$S(yx_2)$	$S(yx_3)$	$S(x_1x_2)$	$S(x_1x_3)$	$S(x_2x_3)$
Total.....	107	773,768	7,125,718	132,287	2,879	1,350,280	140,192	19,863	35,980	52,152	5,222
Between lots.....	1	1,227	1,925	16	0·5	1,537	221	−111	385	−247	−27
Error.....	106	772,541	7,123,793	132,271	2,879	1,348,743	139,971	19,974	35,595	52,399	5,249

*$S(y^2)$, $S(x_1^2)$, $S(x_2^2)$, $S(x_3^2)$ refer to the respective sums of squared mean deviations as $S\ (y-\bar{y})^2$.
†$S(yx_1)$ etc. are the corresponding sums of products.

The sum of squares of the observed feed intake is also included in this table for purposes of comparison. It will be noted that degrees of freedom for error is reduced by 3 over that for error in table 1, because of the calculation of the three regression coefficients. The standard error of 6·61 represents the best estimate, from these data, of the effect of the uncontrolled factors upon feed intake.

Using the same regressions already calculated, the adjusted mean feed consumption of each pen is calculated by satisfying the expression:

Corrected mean feed consumption—

$$(Y - \overline{Y}) - b_1 (X_1 - \overline{X}_1) - b_2 (X_2 - \overline{X}_2) - b_3 (X_3 - \overline{X}_3)$$

in which

Y, X_1, X_2 and X_3 are the actual means of feed consumption, body weight to maintain, weight production of eggs and gain in body weight, respectively, and $\overline{Y}$, $\overline{X}_1$, $\overline{X}_2$, $\overline{X}_3$ the general means of the equivalent characters. Substitution from the data of tables 6 and 7 (appendix A) gives a corrected mean feed consumption as follows:—

Pen A — 809·5 grams
Pen B — 797·5 grams

Appendix B—Table 2

VARIANCE OF FEED CONSUMPTION CORRECTED FOR REGRESSION UPON BODY WEIGHT, PRODUCTION AND GAIN IN WEIGHT

Variance due to:	D/F	Sum of squares	Sum of squares corrected	Variance	S.D.	S.E. for lot means	S.E. for difference between lot means
Total.....	107	773,768	.....	.....	.....	.....	.....
Between lots...	1	1,227	.....	.....	.....	.....	.....
Error.....	103	772,541	242,873	2,358	48·6	6·61	9·35